Draft Interim Report

Evaluation of Environmental Toxicity in the St. Lucie Estuary

Submitted by:

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Introduction

The National Oceanic and Atmospheric Administration's National Status and Trends Program (NS&T) for Marine Environmental Quality has conducted intensive regional surveys to elucidate the incidence, severity, and spatial extent of negative biological effects associated with chemical contamination. These studies are designed to obtain data simultaneously on the levels of chemical contaminants in sediment, results of multiple toxicity tests, analysis of biomarker responses, and changes in benthic community structure. By combining and synthesizing data, these studies provide a holistic understanding of regional environmental quality and the spatial distribution of adverse biological effects related to contamination.

NOAA has performed "biological effect" studies in more than 25 different estuaries and coastal waters throughout the United States. In Florida, NOAA has performed studies in Tampa Bay, Pensacola Bay, Choctawhatchee Bay, St. Andrew, Apalachicola Bay and Biscayne Bay. Recently, NOAA collaborated with the EPA, The Florida Department of Environmental Protection and other state agencies to implement a multi-year study of the quality of estuaries along the coast of the Southeastern United States, including the St. Lucie-Indian River system. The study was implemented through coordination of ongoing state monitoring initiatives and two Federal monitoring programs: NS&T and EPA's Environmental Monitoring and Assessment Program. Two key objectives of this coordinated effort were to assess the condition of estuarine resources throughout the region and to establish a baseline for evaluating changes in the condition of these resources over time.

The overall purpose of the proposed study was to define the environmental conditions in the St. Lucie River, Indian River Lagoon and adjoining ocean waters using the sediment quality triad approach (Long and Chapman, 1985) by addressing the following specific objectives:

1) Evaluate the toxicity of organic sediment extracts using the Microtox™ assay;

2) Evaluate the toxicity of whole sediments using a juvenile clam bioassay; and

3) Evaluate acetylcholinesterase activity in fish and crustaceans as an indicator of exposure to organophosphate and carbamate insecticides.

Methods

Sediment Collection

Sediment samples for clam bioassays were collected from 21 locations throughout the St. Lucie Estuary and Jupiter Inlet (Figures 1 and 2). Sediment samples were collected with a kynar-lined 0.04 m^2 Young grab sampler. Only the upper 2-3 cm of sediment were sampled for use in the toxicity bioassays. Sediments were removed from the sampler with a plastic scoop and transferred to a stainless steel pot. Sediments were then homogenized with a plastic paddle prior to the distribution of the sediments into individual containers for the various bioassays.

Juvenile Clam (*Mercenaria mercenaria*) Assay

Sediments for the clam bioassays were warmed to room temperature and press-sieved through a 212-µm mesh screen. Bioassays were run in pre-cleaned 16-oz glass jars containing 60 ml of sediment and 180 ml of 20 µm filtered seawater. There were five replicates for each sediment sample. Following the addition of the seawater, sediments were allowed to settle under aeration in the bioassay beakers for 24 h before the addition of the clams. After settling, thirty (>212<350 µm) clams were added to each beaker. The bioassays were run at 30‰ salinity, 20°C, and a 12-h light :12-h dark cycle in environmental chambers. Clams in each beaker were fed 5 ml of *Isochrysis galbana* every 48 hours. Temperature, dissolved oxygen, salinity, pH and ammonia were monitored during all bioassays. At the end of ten days, clams were retrieved by re-sieving the sediment through a 212-µm mesh sieve. Clam mortality in each replicate was determined using an Olympus SZH10 microscope under 7.0 x magnification. Site-specific mortality was evaluated in comparison to a reference site (Folly River, SC) using ANOVA and Dunnett's Test (arcsin transformed percent mortality data). Due to the large number of sediment samples to be evaluated, sediments were tested in two separate 10-day assays. A reference sediment (Folly River, SC) sample was included in each of the assays.

Microtox™ Assay

The Microtox™ assay was performed using dichloromethane (DCM) extracts of sediments following the procedures described for South Carolina and Georgia sediments (Long et al., 1998). Extracts were provided to CCEHBR by Columbia Analytical Services, Jacksonville FL.

A suspension of luminescent bacteria, *Vibrio fischeri*, was thawed and reconstituted with deionized water, covered and stored in a 40 ^{0}C well on the Microtox™ analyzer. To assess toxicity, each sample was diluted into four test concentrations. A total of three replicate analyses were performed for each sediment sample. The percent decrease in luminescence in each concentration relative to the reagent blank was then calculated and used to calculate an EC50 (the sediment concentration causing a 50% reduction in luminescence). EC50 results are reported as mg/ml (corrected for dry weight). Site-specific toxicity was evaluated in comparison to a reference site (North Inlet, SC) using ANOVA and Multiple Comparison Tests as well as a nonparametric Distribution Free approach.

Fish sampling

Five sites (33, 34, 36, 37, 40, 47) in the St. Lucie Estuary (SLE) and Jupiter Inlet were sampled for fish (Figure 2). Fish were collected live using hook and line, wrapped in foil and then frozen immediately in dry ice at the site of collection. The samples were shipped frozen to the National Ocean Service laboratory at Charleston, SC, sorted on ice and identified to species. Mangrove snapper (*Lutjanus griseus*) were collected at all five sites. Irish pompano (*Diapterus olisthostomus*) were collected at SLE sites 33, 34, 37 and 47. Spottail pinfish (*Diplodus holbrooki*) were collected at SLE-37 and -47. Whole brain tissues were removed from each fish, wrapped in foil and placed in a –70°C freezer until assayed for AChE activity. There was one fish brain per sample. Mangrove snapper sample sizes ranged from two from SLE-37 to eight from SLE-36. Irish pompano and spottail pinfish sample sizes were five from all sites. SLE-47 served as the reference site for all species. Fish from the sites were compared within species.

Grass Shrimp Sampling

Five sites (1, 33, 34, 36, 48) in the St. Lucie Estuary and Jupiter Inlet were sampled for grass shrimp (Figure 2). Shrimp were collected live with a dip net from each site in water no more than 1m deep. Shrimp were taken from the net, placed in plastic bags and then frozen immediately in dry ice at the site of collection. The samples were transported to the National Ocean Service laboratory at Charleston, SC, sorted on ice and identified to species (*Palaemonetes intermedius*). The shrimp from only two SLE sites (1, 36) were identified as *P. intermedius*. The sampled sites not yielding *Palaemonetes* sp. had salinities over 30 ‰ which is

beyond the normal tolerance range for these shrimp. The shrimp were then divided into two animals per sample, wrapped in foil and placed in a –70°C freezer until assayed for AChE activity. Grass shrimp sample sizes ranged from 16 at SLE-1 to 19 at SLE-36. Laboratory reared *P. intermedius* were used as a reference population. This population of *P. intermedius* was an F1 generation obtained from shrimp originally collected in Manatee Bay, FL.

Acetylcholinesterase Assay

Acetylcholinesterase activity was measured in pooled whole body tissue samples from grass shrimp and brain tissue from individual fish using a continuous assay procedure modified from the original Ellman method (Ellman et al., 1961). Each fish tissue sample (one brain) was analyzed as described by Fulton (1989) and was homogenized (Potter-Elvehjem glass homogenizer with a Teflon pestle) on ice in 50 mM Tris-HCl buffer (pH, 8.1) at 20 mg/ml. Each grass shrimp sample (two shrimp) was analyzed as described by Key et al. (1998) and was homogenized as for fish except using a Pro Scientific Pro 200 motor with a 20 mm x 150 mm stainless steel generator for 45 seconds and then using a Corning 15 ml glass tissue grinder for 45 seconds. Next, 75 µl of each homogenate was added to a test tube containing 1.425 ml of Tris-HCl buffer. After a 15-min incubation period at 30°C, 967 µl of the dilute homogenate was added to a cuvette containing 33 µl of 0.87% 5,5'- dithiobis-(2-nitrobenzoic acid), the color reagent. Finally, 10 µl of 75-mM acetylthiocholine, the substrate, was added to the cuvette then covered with parafilm, inverted to mix, and placed in a spectrophotometer to read continuously for 1 min at a wavelength of 412 nm. For each homogenate sample, three subsamples were assayed. A fourth subsample was incubated with 15 µL of 10 µM eserine to account for nonenzymatic hydrolysis of the substrate. The protein content of the homogenate was determined using the Sigma Assay Procedure, a modification of the original Lowry method (Sigma Chemical Co.1989, Lowry et al. 1951). AChE activity was reported as nmol product formed/mg Protein/min.

The results of the AChE activities were analyzed for normal distribution (Kolmogorov-Smirnov Test) and homogeneity of variance (Bartlett's Test). Statistical analysis of the results was evaluated using ANOVA and Dunnett's Multiple Comparison Test (Gad and Weil 1988). Alpha for all tests was set to 0.05. For the grass shrimp, statistical analyses used the laboratory reared

shrimp as the control group. For the fish, SLE-47 was used as the reference site and all fish were compared within species.

Results

Clam Bioassay

Mortality in juvenile clams exposed to St. Lucie Estuary sediments is shown in Table 1. Mortality ranged from <1% in sediment from SLE-22 to 40% in sediment from SLE-3. All sites with the exception of SLE sites 9, 22, 36 and 48 had significantly lower clam survival than the reference sediment. Eight of the SLE sites (3, 5, 12, 14, 19 27, 33, 37) had more than 15% mortality.

Mean ERM (effects range-median) Quotients (Table 2) were calculated for the sediment collected at each St. Lucie Estuary site. The mean ERM Quotient is an indicator of overall chemical contamination as described by Hyland et al. (1999). Mean ERM Quotients for St. Lucie sediments ranged from 0.002 at SLE-34 to 0.167 at SLE-5. Hyland et al. reported that mean ERM quotients >0.020-0.058 are associated with a moderate probability of degraded benthic communities while ERM quotients >0.058 are associated with a high probability for a degraded benthos. Of the eight sites with more than 15% mortality from the clam assay, five had mean ERM Quotients >0.020. In addition to the compounds included in the calculations of the mean ERM Quotients, other contaminants were measured in many of the sediment samples. These included pesticides such as chlordane, endosulfan and chlorpyrifos.

Microtox™ Assay

Microtox™ results for the St. Lucie Estuary sediments are provided in Table 3. Sediment from only one site (SLE-5) was significantly more toxic than the reference sediment. This site had a mean ERM Quotient of 0.090. Five compounds were detected in sediments at this site at concentrations greater than the ERL (Table 2). Additional contaminants detected at SLE-5 included chlordane, endosulfan and chlorpyrifos.

Acetylcholinesterase Assay

Results from the grass shrimp AChE assay yielded an average acetylcholinesterase level of 24.67 nmol/mgP/min from SLE-36, 31.03 nmol/mgP/min from SLE-1 and 55.29 nmol/mgP/min from the controls (Table 4). Both SLE sites 36 and 1 were significantly lower in AChE level than the controls. These sites were also located at canal mouths. SLE-36 was at the mouth of C-23 canal and SLE-1 was at C-24 canal. Water quality data from these two canals, collected along with the shrimp and fish samples, indicated the presence of carbamate and organophosphate insecticides. These insecticides are known inhibitors of the AChE enzyme in fish and shrimp (Fulton 1989; Key et al. 1998).

Results from the fish AChE assay did not yield any values that were significantly different from the controls (Table 5). Mangrove snapper AChE ranged from 359.09 nmol/mgP/min at SLE-36 down to 214.17 nmol/mgP/min at SLE-34. Irish pompano AChE levels ranged from 431.58 nmol/mgP/min down to 366.80 nmol/mgP/min at SLE-34. Spottail pinfish AChE levels were 294.27 nmol/mgP/min at SLE-37 and 247.8 nmol/mgP/min at the reference SLE-47.

References

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Figure 1. Sediment sample collection locations in St. Lucie Estuary, FL

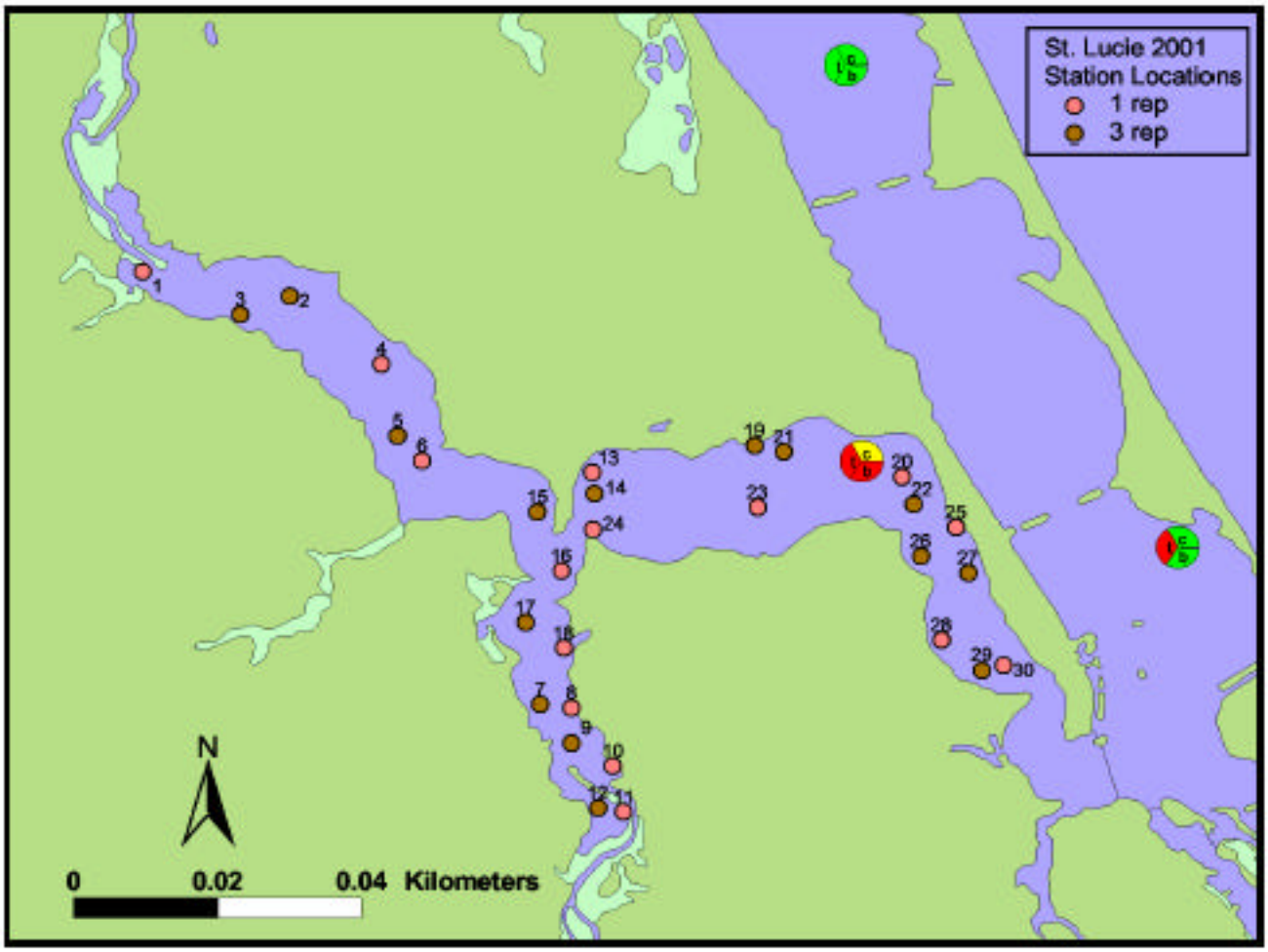

Figure 2. Fish and shrimp sample collection locations in St. Lucie and Jupiter Inlets, FL

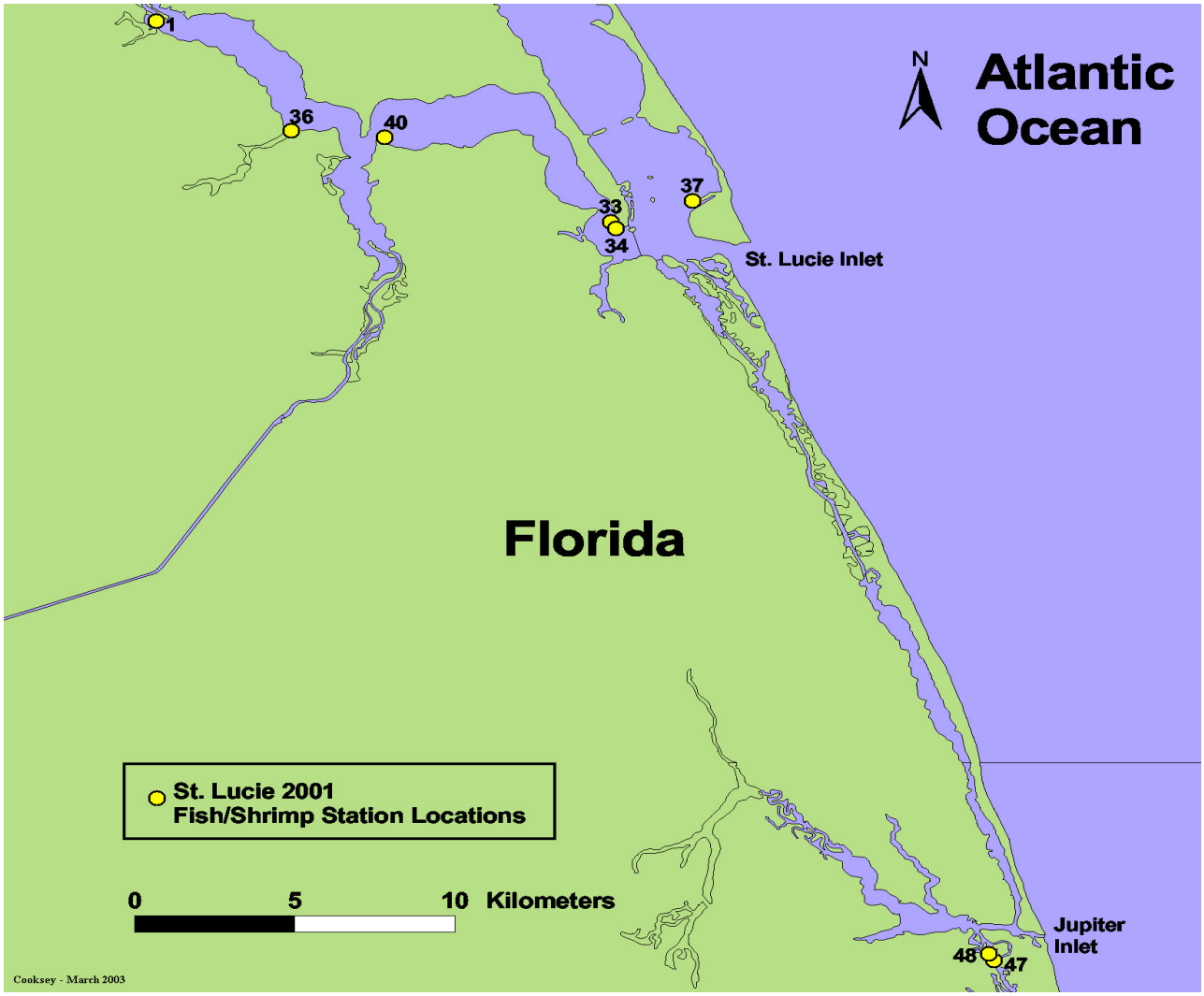

Table 1. Percent mortality in juvenile clams exposed to St. Lucie Estuary sediments.

Site	Mean Mortality (%)	Significantly different (S) from reference
SLE-02	6.0	S
SLE-03	39.3	S
SLE-05	29.3	S
SLE-07	8.0	S
SLE-09	2.7	
SLE-12	33.3	S
SLE-14	15.3	S
SLE-15	11.3	S
SLE-17	10.0	S
SLE-19	16.0	S
SLE-21	12.0	S
SLE-22	0.7	
SLE-26	6.0	S
SLE-27	24.0	S
SLE-29	7.3	S
SLE-33	24.7	S
SLE-34	10.7	S
SLE-36	2.0	
SLE-37	15.3	S
SLE-38	1.1	
SLE-48	14.0	S
SLE-FR (Folly River Reference)	0.0	

Table 2. Mean ERM (effects range-median) quotients and ERL (effects range-low) exceedances in St. Lucie Estuary sediments.

Site	Mean ERM Quotient	ERL Exceedances
SLE-02	0.083	5
SLE-03	0.167	4
SLE-05	0.090	5
SLE-07	0.081	4
SLE-09	0.017	1
SLE-12	0.081	4
SLE-14	0.055	3
SLE-15	0.104	4
SLE-17	0.090	6
SLE-19	0.127	8
SLE-21	0.051	2
SLE-22	0.048	2
SLE-26	0.031	2
SLE-27	0.025	0
SLE-29	0.044	2
SLE-33	0.006	0
SLE-34	0.002	0
SLE-36	0.048	1
SLE-37	0.004	0
SLE-38	0.003	0
SLE-48	0.004	0

Table 3. Microtox toxicity in St. Lucie Estuary sediment extracts.

Site	Mean EC50 (%)	Mean EC50 (mg/ml) dry weight	Comments
SLE-02	>50	>0.747	EC50 >50, presumed non-toxic
SLE-03	>50	>0.759	EC50 >50, presumed non-toxic
SLE-05	28.047	0.540	Significant difference from reference
SLE-07	>50	>1.000	EC50 >50, presumed non-toxic
SLE-09	19.473	1.219	No significant difference from reference
SLE-12	>50	>0.820	EC50 >50, presumed non-toxic
SLE-14	>50	>1.821	EC50 >50, presumed non-toxic
SLE-15	>50	>1.080	EC50 >50, presumed non-toxic
SLE-17	>50	>0.886	EC50 >50, presumed non-toxic
SLE-19	>50	>1.038	EC50 >50, presumed non-toxic
SLE-21	>50	>1.394	EC50 >50, presumed non-toxic
SLE-22	16.270	0.583	No significant difference from reference
SLE-26	>50	>1.555	EC50 >50, presumed non-toxic
SLE-27	>50	>3.029	EC50 >50, presumed non-toxic
SLE-29	>50	>1.912	EC50 >50, presumed non-toxic
SLE-33	>50	>1.515	EC50 >50, presumed non-toxic
SLE-34	>50	>3.962	EC50 >50, presumed non-toxic
SLE-36	38.697	1.435	No significant difference from reference
SLE-37	>50	>3.537	EC50 >50, presumed non-toxic
SLE-38	>50	>4.076	EC50 >50, presumed non-toxic
SLE-NI (North Inlet Reference)	>50	>3.740	EC50 >50, presumed non-toxic

Table 4. Acetylcholinesterase levels and statistical significance in grass shrimp collected from St. Lucie Estuary.

Species	Site	Mean AChE (nmol/mgP/min) [standard error]	Significantly different (S) from control
Grass Shrimp (*Palaemonetes intermedius*)	SLE-01	31.03 [3.51]	S p = 0.0006
	SLE-36	24.67 [2.38]	S p = 0.0006
	SLE-CTL (Lab Control)	55.29 [11.55]	--

Table 5. Acetylcholinesterase levels and statistical significance in fish collected from St. Lucie Estuary.

Species	Site	Mean AChE (nmol/mgP/min) [standard error]	Significantly different (S) from control
Mangrove Snapper (*Lutjanus griseus*)	SLE-33	354.98 [32.72]	--
	SLE-34	214.17 [31.98]	--
	SLE-36	359.09 [33.32]	--
	SLE-37	250.90 [70.13]	--
	SLE-40	217.28 [19.75]	--
	SLE-47 (Control)	345.16 [51.11]	--
Irish Pompano (*Diapterus olisthostomus*)	SLE-33	377.73 [49.81]	--
	SLE-34	366.80 [39.73]	--
	SLE-37	431.58 [55.14]	--
	SLE-47 (Control)	382.47 [45.81]	--
Spottail Pinfish (*Diplodus holbrooki*)	SLE-37	294.27 [31.92]	--
	SLE-47 (Control)	247.80 [51.74]	--